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Persistence of *Metarhizium flavoviride* and Consequences for Biological Control of Grasshoppers and Locusts

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Abstract: The residual infectivity of an oil formulation of the fungal entomopathogen *Metarhizium flavoviride* was measured during a field trial against the rice grasshopper, *Hieroglyphus daganensis*, in north Benin.

The pattern of infectivity was shown to decline exponentially following application, with a half-life of 6·8 days. In this environment, infections due to residual spores from the spray were identified as a key route of infection and accounted for 40–50% of the total infection measured 12 days after application.

To examine the within- and between-season consequences of such residual infection, a simple host–pathogen model was developed. The model revealed that even very small increases in residual activity could provide large increases in total mortality and that under certain conditions, residual infection was essential for effective pest control. This aspect of the activity of mycopesticides is rarely considered.

The implications of these results are discussed in the context of developing optimum spray strategies for locust and grasshopper control under different ecological conditions.

Key words: Mycopesticide, residual infection, entomopathogenic fungus, population dynamics, biological control, locusts and grasshoppers

1 INTRODUCTION

For many years the control of locusts and grasshoppers has centred on the use of synthetic chemical insecticides.[1,2] The most effective chemicals are those with persistent spray residues (spray residue defined as the traces of active ingredient that remain on the soil and vegetation surfaces after the spray aerosol has landed) since they do not require direct contact between spray droplets and target insect for a lethal dose to be acquired.[1,2] However, since the mid-1980s the use of potentially ecologically hazardous persistent chemicals by locust and grasshopper control programmes has not been supported by donor agencies and control programmes now rely on non-persistent chemicals.[1–3] These have virtually no residual activity (e.g. the most widely used insecticide for locust and grasshopper control in recent years is fenitrothion which has a half-life of <24 h[4]) and are much less effective, so that control is often not possible without extensive blanket sprays and repeated applications within a single season.[1] This results in increases in cost of the control programme, damage to the environment and risk to human health and livestock.[1–5]

Because of these problems, there is now considerable interest in biological control, particularly microbial control, as an alternative to chemical spraying (for recent reviews see Prior & Greathead,[6] Streett & McGuire,[7] Bidochka & Khachatourians,[8] Lomer & Prior,[9] Prior & Streett[2]). For example, oil-based mycopesticides containing the fungal pathogen *Metarhizium flavoviride* Gams & Rozsypal (Deuteromycotina:

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Hyphomycetes), are being developed and tested.[10–12] The term mycopesticide is defined here as a pesticide in which the active ingredient is a fungal biocontrol agent rather than a chemical as in most conventional pesticides. *M. flavoviride* acts like a chemical pesticide through direct contact, and field trials with oil-formulated spores have demonstrated effective control of both locusts (C. Kooyman & I. Godonou pers-comm.) and grasshoppers[12–14] under natural field conditions. In one field trial in which three 4-ha plots of savannah were treated against the rice grasshopper, *Hieroglyphus daganensis* Krauss, in north Benin, an 80% reduction in grasshopper density was measured 14 days after a spray application.[14] In this case, the mortality caused by direct impact of the pathogen during spray application was estimated to be only 48%, leading the workers to conclude that secondary pick-up of spores from the vegetation was an important route of infection.

The aim of this paper is to quantify the importance of infection *via* the spray residue on the overall impact of a mycopesticide application. To do this a simple host–pathogen population dynamic model was used, based on measured parameters and supported by field observations, to examine the extent of infection caused by residual spores and the predicted effects of increasing activity of the residue.

2 EXPERIMENTAL METHODS

2.1 Study system

Experimental investigations were conducted in 1994 at field sites near Malanville in north Benin. This area is well developed agriculturally and represents an environment typical of the Sahelian Niger flood plain. *Hieroglyphus daganensis* is one of the most abundant grasshopper species in this area and was the focus of the experimental investigations.

Like most Sahelian species, *H. daganensis* has just one generation per year. Nymphs emerge during the wet season (June–September) from eggs laid the previous year and pass through five or six instars to adulthood in four to six weeks. Reproduction and oviposition takes place over a similar period of about four to eight weeks and then the adults die. Eggs remain in diapause in the soil throughout the dry season until the onset of the rains.[15] During this period the eggs are not susceptible to the pathogen. Therefore, any interaction between grasshopper and pathogen is restricted to the wet season, a period of approximately four months.

For the spray residue experiments, the mycopesticide was applied to three 8-ha plots of natural grassland savannah vegetation (large flat areas of dense grass interspersed with ponds fringed with reeds, cultivated areas and occasional shrubs). Areas such as these, adjacent to farmers' fields, usually support the majority of the grasshopper populations early in the season.[16] Because of difficulties in finding relatively uniform, accessible areas that were suitable for spraying, the replicated plots were positioned at three different villages along the Niger river in the Malanville area: Bodjecali, Madecali and Birni Lafia. These sites were very similar with respect to vegetation and grasshopper densities. Each site contained an 8-ha control plot (separated from the treated plot by at least 200 m), which received no spray treatment.

2.2 Measurement of spray residue

Spores of *M. flavoviride* (IMI 330189) in kerosene + peanut oil (70 + 30 by volume) were applied using hand-held Micro-Ulva sprayers (Micron Sprayers, Ltd. Bromyard, UK) at 2 litre ha^{-1}, giving 5×10^{12} spores ha^{-1}. The persistence and infectivity of the spray residue were monitored using a field bioassay technique similar to that described in Carruthers *et al.*[17] Five field cages ($0{\cdot}5\ m^2 \times 0{\cdot}4$ m high with metal mosquito mesh sides (1-mm mesh), a removable top and an open bottom) were placed within the central areas of the each of the sprayed plots 2 h after treatment (i.e. while the spray residue was still very fresh but after any small droplets should have settled). Any naturally occurring grasshoppers and any predators were removed from the cages by hand. Twenty *H. daganensis* nymphs (predominantly L4, although with some L3 and L5) collected from the unsprayed control plots at each site were then introduced into each cage. After 72 h the grasshoppers were removed from the cages and were incubated in the laboratory for 21 days. During this period any mortality and any *Metarhizium* infections were recorded. Only insects which showed positive mycosis were used to estimate levels of infection. All dead insects were removed from the incubation containers daily, so that the only source of inoculum was from the initial spray application. It was intended to repeat this procedure seven times at each site so as to monitor the spray residue up to 21 days. However, unusually heavy rains caused extensive flooding in the area and it was only possible to collect a full data set (i.e. introductions of untreated insects into cages at 0, 3, 6, 9, 12, 15, 18 and 21 days after treatment) at Madecali which was the last site to flood. Data were collected until day 15 at Bodjecali and until day 12 at Birni Lafia. On each introduction date, the cages were moved to a previously undisturbed position to minimise the effects of the cages on treatment decay. Sweep net samples of 50 grasshoppers were also collected from sprayed and unsprayed areas on each date and were

incubated in the laboratory to assess percentage infection of the resident field populations.

2.3 Population dynamic model

The within-season component of this model begins after egg-hatch, with a population of susceptible grasshopper hosts of density H. A spray event results in a proportion of these becoming infected (spraying is assumed to take place on day 30, when the majority of hosts are at the two- to four-instar stage, since this is stage at which chemical pesticides would normally be applied). This proportion depends on spray efficiency, which is a composite function describing the probability of infection *via* direct contact with the spray, S, and the probability of infection from the spray residue over the remaining 90 days of the season, r.

For the interval between seasons, we assume that the host population grows at a finite rate of increase, F, where those individuals available to breed are the fraction of susceptible hosts that survived the spray application. Density-independent mortality of the grasshoppers is built into the system *via* this finite rate of increase. Density-dependent mortality is ignored on the grounds that in a control programme it is likely that numbers would be maintained below those at which this becomes important.[18] It is further assumed that the pathogen is lost from the system at the end of the season and that no horizontal or vertical infections occur.

Thus, let $H_i(t)$ = the population of healthy grasshoppers in season i, t days after spraying and the number that escape direct spray contact, $H_i(0) = (1 - S) \times$ (healthy population in season i before spraying).

We assume that the instantaneous risk of infection per healthy host per day as a result of contact with the spray residue is described by the negative exponential $r = P \exp(-\alpha t)$, where P is a measure of the initial infectivity of the residue and α is a measure of decay rate (see later and Carruthers *et al.*[17] and Jenkins & Thomas[19]). From this expression the rate of change of healthy hosts due to infections from just the spray residue is given by

$$dH/dt = -P \exp(-\alpha t)H. \quad (1)$$

By solving this equation (dividing both sides by H and integrating with respect to time) we can then derive the equation for the healthy hosts surviving at the end of the season:

$$H_i(d) = H_i(0)\exp[P/\alpha(\exp(-\alpha d) - 1)] \quad (2)$$

where d is the duration of the season after spraying.

From eqn (2), the between-season change in host population after reproduction is therefore given by:

$$H_{i+1}(0) = H_i(0)\exp[P/\alpha(\exp(-\alpha d) - 1)]F. \quad (3)$$

3 RESULTS

3.1 Infectivity of spray residues

The results of the experiment to measure infectivity of the spray residue are presented in Fig. 1. This figure reveals that the levels and patterns of infection over the first 12 days were similar at each site. Initial values for the mean proportion of grasshoppers infected were 0·48 at Madecali and Bodjecali and 0·39 at Birni Lafia. By day 12, the proportions infected had fallen to 0·20, 0·19 and 0·08 respectively. For Madecali and Bodjecali, where samples were taken beyond day 12, the levels of infection were then observed to increase. Since, under field conditions, it takes 12–15 days for an infected grasshopper to die and start producing spores,[14] it is likely that these increases in infectivity were due to horizontal transmission from grasshoppers infected by the initial spray application. To quantify the effects of the spray residue only, therefore, infection data from the first 12 days only were used (i.e. the same observation period for each site) and a single estimate of r for the population dynamic model was obtained by fitting an exponential regression to the mean infection data for the three replicate sites (see Fig. 2). This is legitimate, since, although there was variation between sites and the number of replicates was low, analysis of variance revealed no significant difference between mean infectivity of spray residues at the three sites over the first 12 days ($F_{2,8} = 3{\cdot}78$; $P = 0{\cdot}08$). By correcting these data to give risk of infection per day, the expression for the instantaneous risk of infection was calculated to be $r = 0{\cdot}156 \exp(-0{\cdot}102t)$; $R^2 = 0{\cdot}85$; half life of residue = 6·8 days.

Very low levels of natural infection were observed after 21 days incubation for the sweep net insect samples collected from the control plots. Mean percentage infections for the three control sites were 2% on day 0, 0·7% on day 3, 1·3% on day 6, 0% on day 9 and 0·7% on day 12. Disease levels in the field populations in the sprayed plots were much higher, i.e. 36% for insects sampled immediately after spraying (which is effectively the level of infection from direct contact with the spray) rising to a peak of 62% on day 3. Data for all the days are presented in Fig. 3.

3.2 Model simulations

3.2.1 Validation

In Fig. 3 we compare the levels of infection predicted by the model using the estimate of r, with the levels of infection observed in the grasshopper samples collected from the field after spraying. The model shows a reasonable fit with the field data, although it slightly under-estimates the extent of residual infection over the first three days and then over-estimates it subsequently.

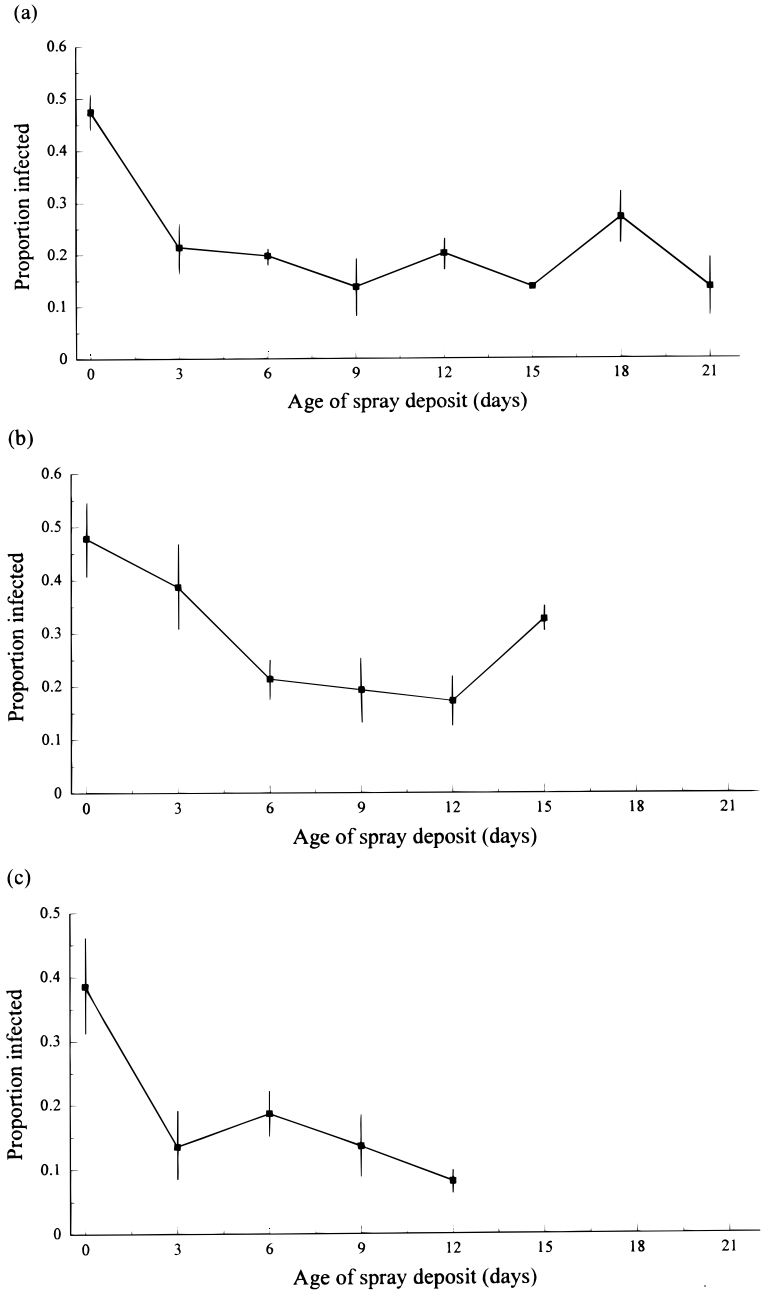

Fig. 1. Mean infectivity of spray residues (±S.E.) measured at three field sites in north Benin following application of oil formulations of *M. flavoviride*. Infectivity was measured by monitoring the proportion of grasshoppers infected after being placed in field cages for three-day periods in the sprayed areas. The field sites were (a) Madecali, (b) Bodjecali, (c) Birni Lafia.

However, the two distributions are not significantly different at the 5% level when compared using a Kolmogorov–Smirnov test ($P = 0{\cdot}329$) (although this test has fairly low power when there are few points in the distributions).

This over-estimation in later observations could be due to the fact that, since only live insects are sampled by sweep netting, cumulative disease incidence in the field is increasingly underestimated as insects treated by the spray application begin to die (either from the disease itself or through interactions with other mortality factors such as predators). Another factor could be immigration of uninfected insects into the sprayed plots and emigration of treated insects out of the plots. However, given that data were collected from the central areas of 8-ha plots and that the estimated mean movement rate of *H. daganensis* is 11 m per day (authors' unpublished data), dispersal is not expected to have had a significant effect during the first 12 days.

This over-estimation due to the expected effects of mortality in the field is corrected by adjusting the model output using the cumulative mortality data from the incubated insects sampled after spraying on day 0 (the assumption being that mortality in these treated

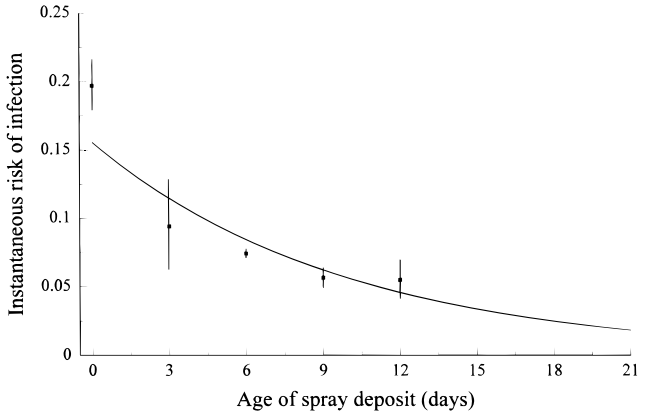

Fig. 2. Mean infectivity of spray residues (±S.E.) pooled for the three replicate sites. The data are corrected to give risk of infection per day and an exponential regression fitted to give the instantaneous risk of infection, r, from contact with the spray residue ($r = 0{\cdot}156 \exp(-0{\cdot}102t)$; $R^2 = 0{\cdot}85$; half life of residue = 6·8 days).

populations held in the laboratory is representative of mortality of treated populations in the field). These data give average mortalities for the three sites of 0% on days 0 and 3, 12% on day 6, 22% on day 9 and 30% on day 12. Multiplying the predicted levels of infection for each day by the proportion of the population expected to be alive on these days (e.g. 1 × 0·36 for day 3, 0·88 × 0·57 for day 6 etc.) greatly improves the fit and the significance level of the prediction ($P = 0{\cdot}82$), revealing that the model provides a good estimate of the effects of the spray residue on overall disease levels averaged across the three sites (Fig. 3). It should be noted that this is not an adjustment of the model *per se* (i.e. the model does predict very well the total number of grasshoppers that become infected from direct and residual contact with the spray) but is simply an adjustment of the output to bring it in line with what is actually measurable in the field (i.e. if grasshoppers are dying then they are not available for sampling and total cumulative infection cannot be measured). It should also be noted that the data used to parameterise the basic model are independent of those used in validation.

3.2.2 Within-season effects

Having obtained a satisfactory estimate of r, we can now use eqn (2) to examine the consequences of residual infection on total mortality by the end of the season for a single spray application. Moreover, by using different parameter values for r we can also investigate the consequences of different residual infection profiles. This is reasonable given that the measured estimate of r reported in Carruthers *et al.*[17] gave a higher initial risk of residual infection (0·224 compared with 0·156) but a more rapid decay (half-life of 2·2 days compared with 6·8 days) than the profile presented here. The reasons for these differences are not clear, although differences in application rate, vegetation density, rainfall and cloud cover between years and sites are all likely to play a role. What is important is that the infectivity of the spray residue is variable and could possibly by manipulated. In Fig. 4(a), therefore, we examine the total mortality from a spray application in relation to direct spray contact rate, for a range of spray residues with different half-lives but with the same initial risk of infection of 0·156. In Fig. 4(b) we do a similar analysis but vary the initial infectivity of the spray residue and keep half-life constant at 6·8 days. These figures reveal that even small increases in half-life or initial infectivity of a spray residue can contribute significant additional mortality by the end of the season. For the highest values of $T_{1/2}$ and P this additional mortality results in excellent overall mortality for even very low initial spray contact rates. This relative contribution of the spray residue declines as spray hit rate increases (if the spray contact rate is 100% there is

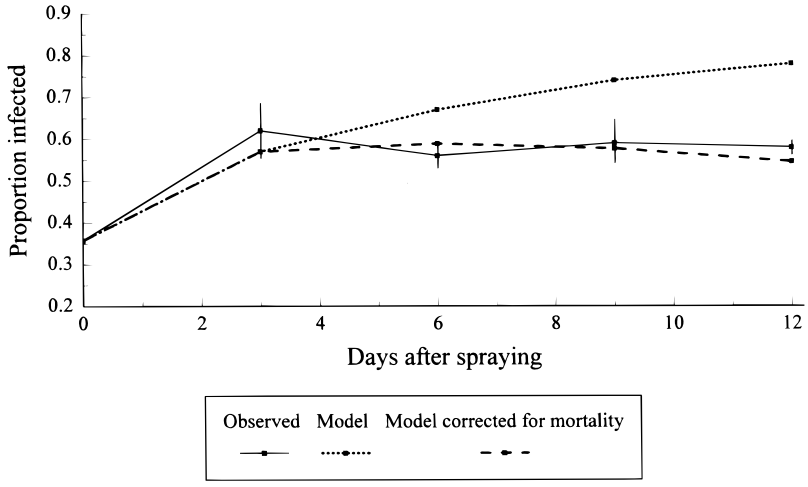

Fig. 3. Changes in disease incidence following application of the mycopesticide for the observed data (mean for the three sites ±S.E.), the basic model output and the model output corrected for grasshopper mortality estimated from the cage incubation samples.

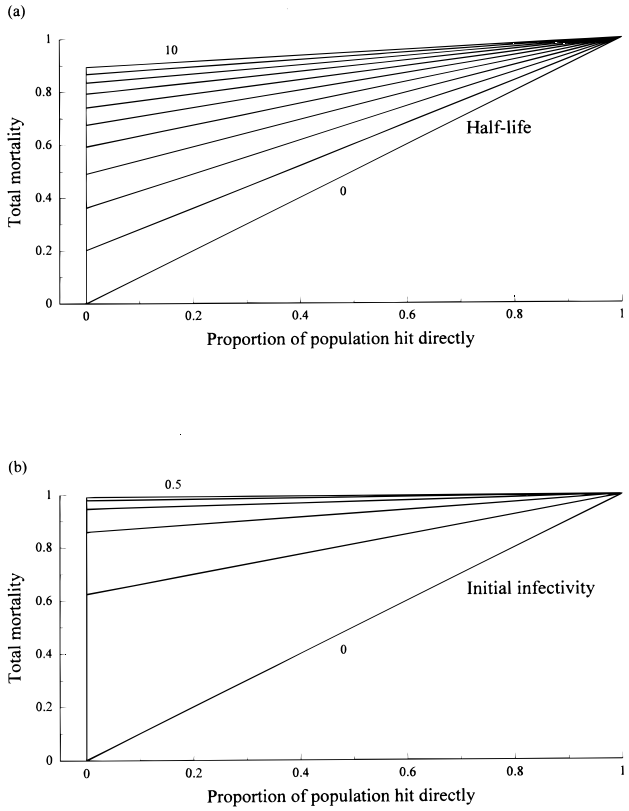

Fig. 4. (a) The effect of persistence of a spray residue on total mortality after 90 days with respect to direct initial spray contact. The different lines show increases in the half-life of the spray residue from 0 to 10 days (shown in increments of one day) with the initial risk of infection set at $P = 0{\cdot}156$. (b) As for (a) except the different lines show increases in the initial risk of infection of the spray residue from 0 to 0·5 (shown in increments of 0·1) with the half-life set at $T_{1/2} =$ 6·8 days.

nothing to be gained through residual infection). For the measured spray residue (which in this range of parameter values has intermediate persistence and initial infectivity) an initial spray contact rate of 50% actually results in approximately 90% mortality, a considerable increase over that for a spray with no residual activity.

3.2.3 *Between-season effects*

Using eq (2) we can examine the long-term consequences of residual infection on grasshopper population dynamics. To do this we need an estimate of the finite rate of increase, F, of the grasshopper population and we need to define a spray control strategy. Unfortunately there are few estimates of F for Sahelian grasshoppers. However, population dynamic studies of *Zonocerus variegatus* (L.),[20, 21] a key pest species in subtropical Africa but also extending into the Sahelian zone,[15] have suggested a range of values up to $F = 10$, so an intermediate value of $F = 5$ was selected for the model. Similarly, there are no good estimates of economic threshold densities of spray action thresholds. For the sake of the model we assume the population is sprayed whenever it exceeds a threshold density of 10 grasshoppers m^{-2}, an arbitrary figure representing intermediate to high grasshopper densities.[18] We also assume that spraying occurs only once a year.

In Fig. 5 we present contour plots of spray frequency as a function of direct spray contact rate and either half-life of the residue or initial risk of infection. The contours represent the number of times the pathogen is sprayed per year (averaged over many generations) in response to the grasshopper density exceeding the spray

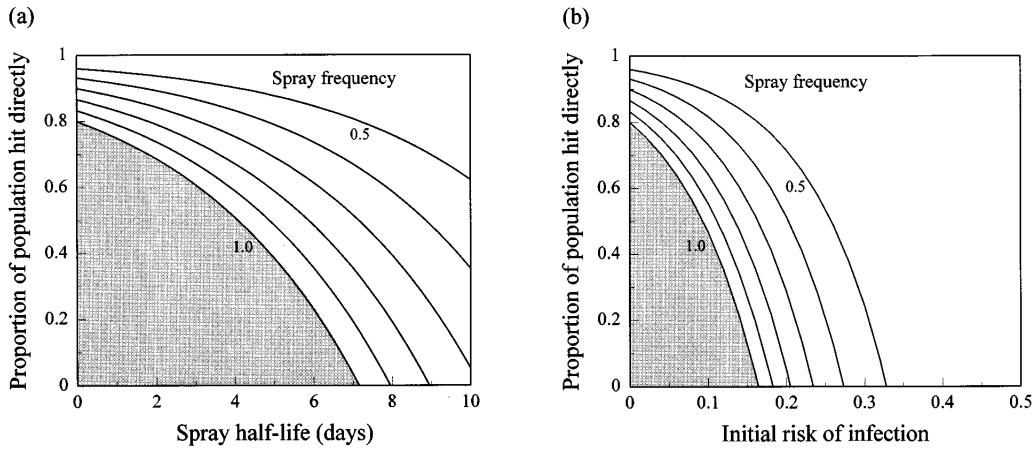

Fig. 5. Predicted frequency of spraying with a *M. flavoviride*-based mycopesticide as a function of the direct contact rate of the spray and either (a) half-life of the spray residue with initial risk of infection set at $P = 0{\cdot}156$ or (b) initial risk of infection with half-life set at $T_{1/2} = 6{\cdot}8$ days. Spraying occurs when the grasshopper density is above a spray action threshold of 10 m^{-2}. The different lines show increases in spray frequency (average number of sprays per year) from 0·5 to 1·0 (shown in increments of 0·1). The shaded region indicates parameter combinations where spray frequency exceeds once per year. Mean finite rate of increase of the grasshopper population is $F = 5$.

threshold. Spray frequencies higher than 1 represent parameter combinations where the pest population always exceeds the spray threshold, i.e. spraying would be required more than once a season to effect control. This region is represented by the shaded area in the figures.

Figure 5(a) clearly shows that, for sprays with very low half-lives, high spray contact rates are necessary for effective control. Under these conditions, small additional increases in spray contact rate result in relatively large reductions in spray frequency. For low to intermediate spray contact rates, control is only possible with sprays with long residual activity. For sprays with little residual infection, repeated spraying within a season is required.

A similar pattern is revealed in Fig. 5(b) for variation in the initial risk of infection. However, the spray frequency appears more sensitive to changes in initial infectivity than changes in persistence since a small proportional increase in the initial infectivity has a relatively greater effect on reducing spray frequency (and hence greater impact on long-term pest control) than an equivalent proportional increase in persistence.

4 CONCLUSIONS AND DISCUSSION

The results of this study demonstrate that infection *via* contact with the spray residue can contribute substantially to the total mortality of grasshopper populations following a spray application of *M. flavoviride*. For the spray trial conducted in north Benin in 1994, $>50\%$ of the predicted total infection and $>40\%$ of the observed infection in field-collected samples was attributable to contact with residual spores. This study also reveals that the extent of this mortality is determined by both the persistence and initial infectivity of the spray deposit; altering either of these by even small amounts can have a large effect on mortality within a season and on long-term pest population dynamics.

Although the models used to identify these results were extrapolated from a single field study, they do not themselves rely on further field data since they only aim to identify what consequences different levels of persistence should have. The principal assumption in these models is that the pattern of infection from a spray residue follows a negative exponential. Not only is this a biologically reasonable model but several other field-based studies have identified this to be the case (e.g. Carruthers *et al.*,[17] Jenkins & Thomas[19]). These other studies also reveal that the level of residual infection is variable and that both persistence and initial infectivity may be manipulated. These are important factors with respect to the economics of mycopesticide use and implementation of a control programme. For example, in systems where direct contact between spray and insect is limited (such as the rice grasshopper system described here, where a combination of high, dense grass vegetation together with the behavioural escape response of the host prevented high initial contact rates), contact with residual spores is essential for effective control. Hence, maximising residual infectivity through increases in persistence and/or initial infectivity is likely to have a significant impact on the cost : benefit ratio of a control programme. In contrast, under conditions where direct contact between spray and insect is not limiting, or where prolonged activity of the spray residue is considered undesirable as in some conservation areas, for example, efforts to maximise efficiency of the spray application will be of greatest benefit (which could include increasing the initial infectivity of the residue but not the persistence). Thus, understanding the mechanisms which influence the pattern of residual infectivity is an important consideration in the development of optimum spray strategies.

Laboratory investigations on the study isolate of *M. flavoviride* (IMI 330189) have revealed that as little as 1 h exposure to simulated solar radiation can dramatically reduce conidial viability and it is expected that a single day of direct sunlight would cause complete inactivation.[22,23] Several other authors have shown that sunlight and artificial sources of ultraviolet radiation are highly damaging to conidial stability (e.g. Ignoffo *et al.*,[24] Alves *et al.*,[25] Daoust & Pereira,[26] Carruthers *et al.*[27]). As such, Scherer *et al.*[22] suggest that, for spray residues to be important for locust and grasshopper control, the pathogen must contact a mobile host within 24–48 h. However, even for the residue measured in 1992,[17] less than 50% viability was lost over the first 48 h and measurable infection was recorded after 10 days. For the residue measured in the course of the present trials in 1994, a half-life of 6·8 days resulted in persistence for considerably longer. Thus, under natural field conditions, spores appear to remain active in protected positions in the environment (e.g. within the vegetation canopy). The extent to which this can be manipulated is unclear but the differences between 1992 and 1994 indicate considerable potential. The levels of infection from spray residues presented in a study of Jenkins & Thomas[19] reveal that the initial infectivity, P, of different residues may also vary considerably (values up to $P = 1{\cdot}6$ were measured).

Although unquantified, the 1994 season was characterised by unusually high rainfall which increased vegetation density and cloud cover (reducing UV radiation) and reduced temperature. These factors are likely to have improved pathogen survival. Achieving equivalent alterations in pathogen survival through modifications to formulation (e.g. use of solar protectants[23]), application (e.g. alterations in dose and droplet size[19]) and selection of pathogen isolate, together with the influence of major abiotic and biotic factors that differ between ecological zones, requires further examination.

As mentioned in the introduction, the non-persistent chemical pesticides currently available for use in locust and grasshopper control are generally less effective than persistent chemical alternatives. This is particularly so in treatment of mobile hopper bands and restricted roosting sites (which are only occupied temporarily) where secondary uptake of chemical pesticides can be of great importance.[28] The results of the present study suggest that in some circumstances, the mycopesticide could have a similar advantage over chemicals with very short half-lives. That the persistence of the residue can be extended to the levels of chemicals such as dieldrin is unlikely (half-life is strongly influenced by soil and climatic factors but values may range from 30 to >100 days[29,30]). Thus, strategies for locust control such as band spraying which require prolonged persistence of the residue to be effective (see Prior & Streett[2] for a recent review of pesticide strategies for locust and grasshopper control), are likely to remain impractical. However, a recent study by Thomas *et al.*[18] has identified the possibility for enhanced persistence of the pathogen after spraying through production of new spores in infected cadavers. Under conditions where this is possible, secondary pick-up, together with cycling of the pathogen through horizontal infection, could enable the effects of a single mycopesticide application to last for many weeks. This should increase the opportunities for biological pest control using pathogens.

As a final point, mobile insects such as grasshoppers and locusts are notoriously difficult to sample and, as such, obtaining quantitative data on the efficacy of a slow-acting mycopesticide has many problems (e.g. see Lomer *et al.*[14]). In the field trial reported here, laboratory incubation of insects sampled at various times after spraying revealed high percentage levels of infection. Unfortunately, these results alone do not provide any evidence of mortality in the field where incubation conditions are very different and where behavioural responses of infected grasshoppers may alter the pattern of disease progression.[31] However, the good fit between the observed pattern of disease incidence and that predicted by the host–pathogen model when mortality was included, does suggest that mortality in the field corresponded closely to that monitored in the incubation containers. Thus, even in the absence of reliable estimates of grasshopper densities, the model provides strong evidence for population reductions following applications of the mycopesticide. As such, this study provides a good example of the potential for utilising population dynamic approaches in applied pest management.

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